High-Q™-Spin-Column Bacterial Genomic DNA Purification Kit

Ordering info

TBK0115, 3 reactions (sample)

TBK0116, 50 reactions

TBK0117, 200 reactions

Description

High-Q™ Bacterial Genomic DNA Purification Kit is an optimized kit to purified genomic DNA from bacterial culture. Suitable for Gram negative and Gram-positive bacteria, purification is based on silica spin columns in presence of chaotropic salts. Genomic DNA is obtained in high quantity and quality.

Features

- High yield and purity, 3-20 µg, A260/A280 1.8 ± 0.2; A260/A230 2.0 ± 0.2.
- Scalable, easily to process many samples simultaneously.
- No phenol extraction.
- Fast, easy and cost-effective protocol.

Applications

DNA obtained is suitable for downstream molecular biology applications such as PCR, enzymatic digestion for cloning or Southern, genotyping, etc.

Quality Control

DNA isolation from stationary *E. coli* culture is checked by: integrity (agarose gel electrophoresis), quantity and quality (A260/280 and Abs260/230 = 1.8 ± 0.2).

Storage

- Store the kit at 25°C.
- Store Proteinase K Solution, Lysozyme Solution and RNase Solution at -20°C.

Kit Components

Components	TBK0116	TBK0117
High-Q™ Spin Column with Collection Tubes	50	200
BAC Buffer	12 mL	40 mL
BEC Buffer	15 mL	45 mL
Proteinase K	30 mg [a]	4 x 30 mg
Proteinase K Resuspension Buffer	1.5 mL	4 x 1.5 mL
Lysozyme	30 mg [b]	4 x 30 mg
WB1 Buffer	20 mL[c]	70 mL[e]
WB2 Buffer	8 mL[d]	24 mL[f]
RNase lyophilized	12 mg [g]	2 x 20 mg [h]
RNase Resuspension Buffer	1.5 mL	2x 2 mL
Elution Buffer	15 mL	25 mL

Order Info Kit Components: High-Q™ Spin Column with Collection Tubes *(TBM0010)* | *BAC Buffer (TBB0516)* | *BEC Buffer (TBB0515)* | *Proteinase K (TBZ0305)* | *Lysozyme (TBZ0312)* | *RNase lyophilized (TBZ0318)* | *WB1 Buffer (TBB0511)* | *WB2 Buffer (TBB0512)* | *Elution Buffer (TBB0510).*

¡Components for samples are ready to use!

Before its use:

[a] To prepare a 20 mg/mL solution, spin Proteinase K tube and add 1.5 mL Proteinase K Resuspension Buffer. Store Proteinase K solution obtained in aliquots at -20°C.

[b] To prepare a 50 mg/mL solution, spin Lysozyme tube and add 0.5 mL Water (*Molecular Biology Grade*). Store at -20°C

[c] Add 12 mL absolute ethanol and mix well.

[d] Add 32 mL absolute ethanol and mix well.

[e] Add 42 mL absolute ethanol and mix well.

[f] Add 96 mL absolute ethanol and mix well.

[g] Add 1.2 mL RNase Resuspension Buffer and mix well.

[h] Add 2 mL RNase Resuspension Buffer and mix well. Store RNase A solution at -20°C.

PROTOCOL

1. Transfer 1.5 -2 mL of an overnight bacterial culture in a centrifuge tube.
2. Centrifuge at 13,000 g for 1 minute and completely remove the media supernatant with a pipet tip.
3. Add **200 µL BAC Buffer** and resuspend the pellet completely.
4. Add **10 µL Lysozyme Solution (50 mg/mL)** and ***20 µL RNase (10 mg/mL)*** and mix. Incubate at 37°C, 30 minutes.
5. Add **20 µL Proteinase K.**
6. Add **200 µL BEC Buffer,** mix by vortex vigorously and incubate 30 minutes at 55°C.
7. Add **200 µL Absolute Ethanol** and mix by vortex vigorously.
8. Transfer the **mixture** to a High-Q™ Spin Column placed into a Collection Tube using a pipette.
9. Centrifugate at 13,000 g for 1 minute and discard the flow-through. Ensure that the entire sample mixture has passed into the collection tube; if sample remains in the column, centrifuge again.
10. Place the spin column into the Collection Tube and add **500 µL WB1 Buffer** (✓).

    ✓ *Check ethanol has been added.*
11. Centrifugate at 13,000 g for 1 minute and discard the flow-through.
12. Add **700 µL WB2 Buffer** (✓)

    ✓ *Check ethanol has been added.*
13. Centrifugate at 13,000 g for 1 minute and discard the flow-through.
14. To remove residual ethanol, centrifugate at 13,000 g for 1 minute.
15. Place the spin column into a 1.5 mL tube.
16. Add **50-100 µL Elution Buffer** or **Water, Molecular Biology Grade** (pre-heated at 70°C) on top of the silica matrix. Incubate at room temperature for 2 minutes.
17. Centrifugate at 13,000 g for 1 minute to collect DNA in eluate.
18. Check DNA quality on agarose electrophoresis gel and quantity by spectrophotometry. Store at -20°C.